AMENDMENTS TO THE SPECIFICATION

In the Title:

Please replace the current title with the following new title:

--METHODS FOR INTRODUCING HYDROXYL OR EPOXIDE GROUPS INTO POLYKETIDES USING OleP--

In the Abstract:

Attached hereto as Exhibit A is a substitute Abstract. The Examiner is requested to insert this abstract in place of the current abstract at page 17 of the specification.

Continuity Data:

On page 1, line 5, please replace the present paragraph with the following paragraph:

--This application is a divisional of U.S. Application Serial No. 09/768,927, filed January 23, 2001, now U.S. Patent No. 6,388,099 B2, issued May 14, 2002; which claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application No. 60/177,660, filed January 27, 2000; is a continuation-in-part of Application Serial No. 09/428,517 filed October 28, 1999, now U.S. Patent No. 6,251,636 B1, issued June 26, 2001 which has a PCT counter part International Application No. PCT/US99/24478, filed October 22, 1999; Application No. 09/428,517 in turn claiming the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Applications Nos. 60/120,254, filed February 16, 1999, and 60/106,100 filed October 19, 1998; the disclosures of each of which are incorporated herein by reference.--

Updated Information as set forth in paragraph 8 (a-f) of the Action:

1. The updated information referred to in subparagraph a) on page 1, line 29, has been corrected in the Preliminary Amendment filed on November 16, 2001.

2. On page 2, commencing at line 7, please replace the existing paragraph with the following paragraph:

--The study of oleandomycin biosynthesis has been progressive over the past decade, due largely to the identification and sequencing of several biosynthetic and related genes by Salas and coworkers. Analysis of these gene sequences has revealed enzymes putatively involved in synthesis and attachment of the two deoxysugars, regulatory and antibiotic resistance genes, and a P-450 monooxygenase (see Rodriguez *et al.*, *A cytochrome P450-like gene possibly involved in oleandomycin biosynthesis by Streptomyces antibioticus*, *FEMS Microbiol. Lett.* 127: 117-120, 1995; Olano *et al.*, *Analysis of a Streptomyces antibioticus chromosomal region involved in oleandomycin biosynthesis, which encodes two glycosyltransferases responsible for glycosylation of the macrolactone ring, Mol. Gen. Genet.* 259: 299-308, 1998; and Quiros *et al.*, *Two glycosyltransferases and a glycosidase are involved in oleandomycin modification during its biosynthesis by Streptomyces antibioticus*, *Mol. Microbiol.* 28: 1177-85, 1998, each of which is incorporated herein by reference). Thus, a single open reading frame (ORF) encoding a polypeptide subunit of a type I polyketide synthase was identified and, based on comparison to 6-deoxyerythronolide B synthase (DEBS), was hypothesized to encode the last two modules of the oleandomycin PKS (OlePKS; see Swan *et al.*, *supra*). The cloning, characterization, and sequence determination of the other genes encoding the proteins of the OlePKS are described in PCT patent application no. PCT/US99/24478, incorporated herein by reference.--

3. On page 3, commencing at line 22 and continuing to page 4, line 2, please replace the existing paragraph with the following paragraph:

--Thus, in one embodiment, the present invention provides a method for introducing one or more hydroxyl groups or an epoxide into a polyketide, which method comprises expressing a

recombinant gene encoding a P450 monooxygenase in a host cell. In one embodiment, the P450 monooxygenase is not naturally expressed by the host cell. In another embodiment, neither the P450 monooxygenase nor the polyketide is naturally expressed by the host cell. In a preferred embodiment, the host cell is a *Streptomyces* host cell. In another preferred embodiment, the P450 monooxygenase is OleP. In another preferred embodiment, the polyketide synthase is 6-deoxyerythronolide B synthase or 8,8a-deoxyoleandolide synthase.--

4. On page 4, commencing at line 28, please replace the existing paragraph with the following paragraph:

--The present invention relates to the heterologous expression of the OlePKS coding sequence and/or OleP, the P-450 hydroxylase that converts 8,8a-deoxyoleandolide to oleandolide in *Streptomyces antibioticus*. The OlePKS is encoded by three ORFs - *oleAI*, *oleAII*, and the previously identified ORFB (see Swan *et al., supra*; ORFB is designated *oleAIII*) - that span 35 kb of DNA. Each of the ORFs encodes two PKS modules, as in *eryAI-AIII*, and examination of the active site domains within the modules also reveals an organization similar to the active site arrangement of DEBS, as shown in Table 1, below. The sequence of the OlePKS genes and recombinant vectors from which those genes can be isolated are described in PCT application no. PCT/99/24478, incorporated herein by reference.--

5. On page 5, commencing at line 24, continuing to page 6, line 2, please replace the existing paragraph with the following paragraph:

--In contrast with the loading module of DEBS, which consists of an acyl transferase (AT) domain - that loads a propionate starter unit - and an acyl carrier protein (ACP), the OlePKS loading module has an additional KS-like domain (KSQ) with a glutamine instead of a cysteine at the active site. These domains have been shown to decarboxylate acylthioesters within PKSs and the related fatty acid synthases (see Witkowski *et al., Conversion of a ß-ketoacyl synthase to a malonyl decarboxylase by replacement of the active-site cysteine with glutamine,* Biochemistry, (1999) 38(36):11643-11650; and Bisang *et al., A chain initiation factor common to both modular and aromatic polyketide synthases, Nature* 401: 502-505, 1999, each of which is incorporated herein by

reference). Therefore, the OlePKS is believed to initiate 8,8a-deoxyoleandolide synthesis by loading the ACP with a malonate unit and performing a decarboxylation to generate acetyl-ACP.--

6. On page 6, line 3 through line 15, please replace the existing paragraph with the following paragraph:

--The heterologous production of 8,8a-deoxyoleandolide can be accomplished in *Streptomyces lividans* and *S. coelicolor*. Preferred strains of these organisms are described in U.S. Patent No. 5,672,491 and U.S. patent application Serial No. 09/181,833, filed 28 Oct. 1998, now US Patent No. 6,177,262, incorporated herein by reference. A vector for heterologous expression of the OlePKS was constructed in a manner analogous to those developed for DEBS and the picromycin PKS (PicPKS; see Kao *et al.*, *Engineered biosynthesis of a complete macrolactone in a heterologous host, Science* 265: 509-512, 1994; Tang *et al., Elucidating the mechanism of chain termination switching in the picromycin/methymycin polyketide synthase, Chem. & Biol.* 6: 553-558, 1999; and PCT application no. PCT/US99/11814, each of which is incorporated herein by reference). Plasmid pKOS098-4 is an autonomously replicating SCP2*-based shuttle vector containing the three OlePKS open reading frames downstream of the *S. coelicolor act*I promoter and *act*II-ORF4 transcriptional activator.--

7. On page 11, lines 14 through 25, please replace the existing paragraph with the following paragraph:

--DNA manipulations were performed in *Escherichia coli* XL1-Blue (Stratagene) and DH10B (BRL). *Streptomyces lividans* K4-114 and K4-155, genotypically identical strains which contain deletions of the entire actinorhodin gene cluster, were used as host strains for the production of polyketide compounds (see Ziermann *et al.*, *Recombinant polyketide synthesis in Streptomyces: Engineering of improved host strains, Biotechniques* 26: 106-110, 1999, and U.S. patent application Serial No. 09/181,833, filed 28 Oct. 1998, now US Patent No. 6,177,262, each of which is incorporated herein by reference). *S. lividans* was transformed according to standard methods, and clones were selected with thiostrepton (50 μg/ml) or apramycin (200 μg/ml) overlays on R5

regeneration plates (see Hopwood *et al.*, *Genetic Manipulation of Streptomyces: A Laboratory Manual*, *The John Innes Foundation*, Norwich, U.K., 1985, incorporated herein by reference).--

8. On page 12 of the specification, lines 5 through 17, please replace the existing paragraph with the following paragraph:

--Genomic DNA was isolated from an oleandomycin producing strain of *S. antiobioticus* (ATCC 11891) using standard procedures (see Hopwood *et al.*, *supra*). A genomic library was prepared in Supercos™ (Stratagene) using DNA partially digested with *Sau3*A I following the supplier's protocols. A probe was prepared by PCR amplification of genomic DNA using primers specific to the KS domains of modules 5 and 6 of OlePKS. The genomic library was then probed by colony hybridization with 32P-labeled probe. Cosmids containing the desired DNA inserts were verified by PCR with the same primers and by comparison of restriction digest patterns to known sequences. Two overlapping cosmids, pKOS055-5 and pKOS055-1, were identified which cover approximately 65 kb of DNA and contain the entire oleandomycin gene cluster. See PCT application no. PCT/US99/24478 and U.S. patent application Serial No. 09/428,517, filed 28 Oct. 1999, now US Patent No. 6,251,636, each of which is incorporated herein by reference.--